



“Rare biosphere” plays important roles in regulating soil available nitrogen and plant biomass in alpine grassland ecosystems under climate changes

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ABSTRACT

Ecological roles of “rare biosphere” (RB) were rarely discussed in alpine grassland ecosystems. We explored the roles of RB and specific taxa of soil bacteria and fungi in regulating soil nutrients and aboveground biomass of an alpine meadow and alpine steppe under warming and enhanced rainfall scenarios on the Qinghai-Tibetan plateau. In the context of alpine meadow and alpine steppe, the relative abundance of bacterial RB was ~40% in the bacterial community, and it was < 10% for fungal RB in the fungal community. The relative abundance of the RB of soil bacteria and fungi were not changed by warming or enhanced rainfall. All specific bacterial taxa and over 90% specific fungal taxa belonged to the RB. The RB, including the most specific taxa, explained many more variations in the contents of soil NO₃-N and NH₄-N than the high abundant microbial species under warming and enhanced rainfall conditions. The aboveground biomass of the alpine meadow and steppe were strongly affected by the contents of soil NO₃-N, available phosphorus and total nitrogen under the condition of warming and enhanced rainfall. Our findings suggest that the robust soil microbial RB plays an important role in regulating the content of soil available nitrogen, which could profoundly affect aboveground plant biomass of alpine grassland ecosystems under climate change conditions.

1. Introduction

Biodiversity is critically important in maintaining the stability of ecosystems (McGrady-Steed et al., 1997; Tilman et al., 2006). The loss of biodiversity, especially the loss of some specific and rare species, could reduce the ability of an ecosystem to adapt to environmental changes (Chapin III et al., 2000; Yang et al., 2013; Hua et al., 2014). In past decades, the aboveground biodiversity and its contribution to ecosystem functions have been widely studied (Gaston, 2000). However, the belowground microbial biodiversity, the largest pools of biological diversity on the Earth (Decaëns, 2010), remain poorly understood for most ecosystems until the relatively recent advent of molecular techniques allow for a more thorough and rigorous exploration of the microbial biosphere (Horner-Devine et al., 2004; Rosling et al., 2011). It is well known that soil microbial diversity plays an important role in regulating ecosystem functions by affecting biotic and abiotic elements in both above- and belowground systems (Bardgett et al., 1998; Llorens-Marès et al., 2015).

Previous studies have revealed that the abundance of some rare microbial taxa, the so-called conditionally rare taxa, would occasionally increase and significantly affect microbial communities over time (Shade et al., 2014). Some rare taxa may serve as keystone species, which can strongly affect ecosystem functions (Giovannoni and Stirling, 2005). Some environmental specific taxa play important roles in adjusting the ecosystem to environmental changes (Yang et al., 2013). It is suggested that the RB should be considered as a collective of low-abundance taxa, which can shape ecosystem functions (Lynch and Neufeld, 2015). The microbial RB, according to a few studies on marine or wetland ecosystems, is thought to play a critical role in maintaining the structure and functions of microbial communities and ecosystem stability in response to environmental perturbations (Pester et al., 2010; Gobet et al., 2012; Lynch et al., 2012; Sauret et al., 2014). However, few studies have documented the roles of microbial RB in shaping functions of territorial ecosystems such as alpine grasslands.

The alpine grassland is a key global biome and ecosystem, which contains some of the most unique plants, animals and microbes in the

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world (Bews, 1930). Alpine grasslands, mainly alpine meadow and alpine steppe cover over 60% of the Qinghai-Tibetan Plateau (QTP) (Dong and Sherman, 2015). The soils of QTP's grasslands are characterized by a shortage of available nitrogen and phosphorus, which limited the growth of alpine plants (Dong et al., 2015). The QTP is one of the world's biodiversity hotspots (Myers et al., 2000; Ding et al., 2013), while the biodiversity of alpine grasslands in this region is threatened by multiple drivers including climate changes, i.e., warming and wetting (Chen et al., 2013), and inappropriate grazing management such as overgrazing in recent decades (Harris, 2010). However, there are few studies focusing on the soil microbial RB, which may shape the structure and functions of microbial communities to increase the stability of alpine grassland ecosystems on the QTP in response to environmental stresses such as climate changes.

To explore the ecological roles of the RB of soil bacteria and fungi in regulating the contents of soil available nitrogen and phosphorus under climate change conditions in alpine grassland ecosystems, we examined soil microbial RB as a collective of low abundance taxa of soil bacterial and fungal communities. Our data was collected after three-year stable warming (SW), variable warming (VW) and enhanced rainfall (ER) in an alpine meadow (AM) and alpine steppe (AS) on the QTP. Our hypotheses were: (1) the relative abundance of the RB of soil bacterial and fungal would be changed by warming or enhanced rainfall, i.e., the abundance of soil microbial RB is sensitive to climate changes. And (2) soil microbial RB plays an important role in regulating soil available nitrogen and phosphorus, which would help stabilize the aboveground biomass of alpine grasslands under climate changes.

2. Methods and materials

2.1. Study site and field experimental design

Study site This study was conducted in the central region of the QTP, i.e. Nagqu and Bange counties of Tibetan Autonomous Region (Fig. 1). The annual mean precipitation is 432 mm, the annual mean temperature is -1.2°C (1982–2013) and the agrotype is alpine meadow soils in Nagqu County. It is 330 mm, -0.4°C and alpine steppe soil in Bange County. The alpine meadow (AM) dominated by *Kobresia pygmaea* is the typical vegetation in Nagqu County. The alpine steppe (AS) dominated by *Stipa purpurea* is the typical vegetation in Bange County.

Experimental design In 2010, twelve $4\text{ m} \times 4\text{ m}$ plots in each of the two grassland types, AM and AS with similar topography (two pieces of $\sim 200\text{m}^2$ flat rangeland where slope are less than 5°) and land use histories (both areas in AM and AS were used as summer pasture before the experiment) were fenced to exclude grazing. For each grassland type, three plots were randomly allocated to each of the four treatments: control (CK), stable warming (SW), variable warming (VW) and enhanced rainfall (ER). Conical fiberglass open-top chambers (OTCs) were 1.20 m in diameter at the base, 0.65 m in diameter at the top and 0.45 m high, and mini fans were used to control the temperature (Klein et al., 2007). The temperature in the OTCs of ER treatment was maintained at the ambient temperature outside the OTCs. According to the fourth report of IPCC, global temperature will increase $1.1\sim 2.9^{\circ}\text{C}$ at 2090–2099 when compared with the reference period from 1980 to 1999 (IPCC, 2007). Therefore, the temperature in the OTCs of SW treatment was maintained at a constant temperature of 1.55°C higher than the ambient temperature. There were no mini fans in the OTCs of the VW treatment, and the daily air temperature at 5 cm above the soil surface was unevenly elevated with an average temperature rise of about 2.0°C over ambient temperatures. According to the prediction of precipitation trend in central QTP which conducted by Xu et al (2008) using 40 years' observation data, 20% rainfall collected by pails was added to the OTCs after each rainfall event in the ER treatment. The surface (5 cm belowground) soil temperature was significantly improved and the surface soil moisture was significantly reduced by OTCs during the growing season (from June to August) in the AM and AS

(Ganjurjav et al., 2016).

Sampling In early August 2013, after 3-year treatments, 24 soil samples (0–15 cm) from the AM and AS (there were four treatments, namely CK, SW, VW and ER for each grassland type; three replicates (sub-plots) for each treatment) were selected, sealed in polyethylene bags, stored in an icebox under 4°C and transported to labs within five days for extraction. The aboveground biomass (AGB) of these two types of grasslands with each treatment was collected by cutting the grass swards in three $0.5\text{ m} \times 0.5\text{ m}$ quadrats (replicates).

2.2. Soil microbial sequencing and soil nutrients measurement

2.2.1. Soil microbial sequencing

The 16S rRNA gene (for bacteria) and the ITS rRNA gene (for fungi) were amplified, purified and quantified, separately. A mixture of amplicons was used for sequencing on the Illumina Miseq platform. After a series of data processing, the operational taxonomic units (OTUs) were defined as sharing $> 97\%$ sequence identity using the furthest neighbor method. More details about the sequencing and raw data processing can be found in Zhang et al (2016). The sequence data can be found in the National Center for Biotechnology Information (NCBI) database under the accession number of SRP072264.

2.2.2. Soil nutrients measurement

Soil total nitrogen (TN) was measured using an element analyzer (EA 3000, Italy). The contents of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were measured on a flow injection auto-analyzer (AACE, Germany). Soil total phosphorus (TP) and available phosphorus (AP) were measured using inductively coupled plasma spectrometers (ICP, SPECTRO ARCOS EOP, Germany).

2.3. Statistical analysis

2.3.1. The identification of RB and specific taxa

For both bacteria and fungi communities, the OTUs with less than 0.1% of total community relative abundance was defined as RB (Lynch and Neufeld, 2015). The specific taxa were identified as the OTUs that only tested in some treatments, e.g. the OTUs only detected under the treatment of the SW in the AM.

2.3.2. Variances of indicators among treatments

Two-way ANOVA, using IBM SPSS Statistics software (version 19.0), was conducted to detect the effects of grassland types and experimental treatments on soil TN, TP, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, AP, AGB and OTUs' percentage and the relative abundance of the RB.

2.3.3. Path analysis

To explore the effects of soil microbial RB and specific taxa on soil nutrients including TN, TP, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and AP and the aboveground biomass (AGB), a path analysis was conducted using Amos 21 (IBM, Chicago). The abundance of the RB and specific taxa were used as exogenous variables. The AGB and soil nutrients indexes, including TN, TP, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and AP, were used as endogenous variables. To assess the relative importance of RB in regulating soil nutrients another path analysis, using the abundance of high-abundant bacterial and fungal taxa as exogenous variables, was conducted. According to the explained variations of endogenous variables in these two path models, we could obtain evidences to judge whether the RB has significant effect on the cycling of soil nutrients influentially. All data of the AM and AS under the treatments of CK, SW, VW and ER was used together in these path analyses. A qualified path analysis should be indicated by a non-significant χ^2 test ($P > 0.05$), high comparative fit index (CFI) (> 0.95) and low root mean square error of approximation (RMSEA) (< 0.05) (Jonsson and Wardle, 2010; Wei et al., 2013).

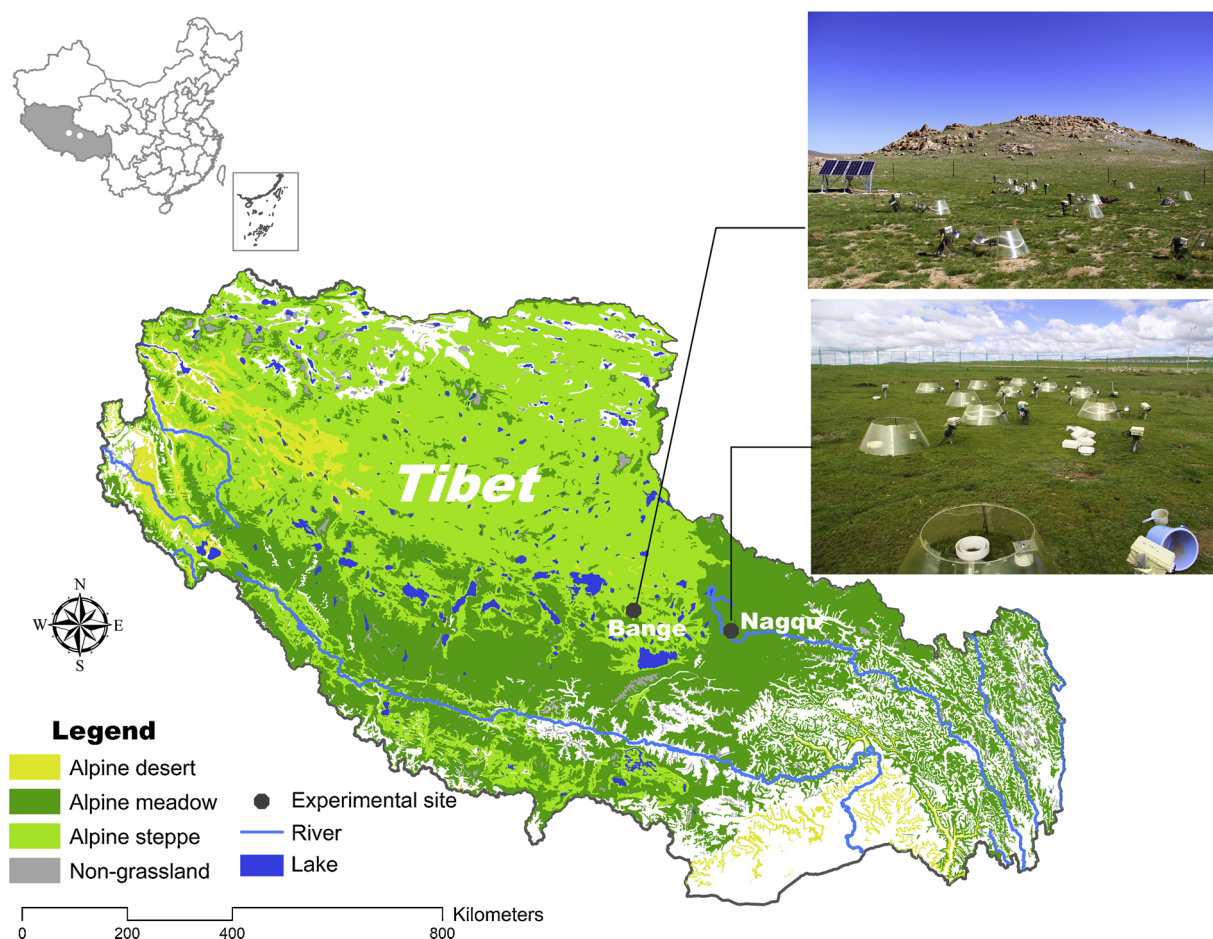


Fig. 1. The location of Bange and Nagqu counties in Tibetan Autonomous Region (the main panel) and photos of experimental plots in Bange and Nagqu.

3. Results

3.1. The RB of soil microbes under climate changes

The OTUs' percentage of bacterial rare biosphere (BRB) was profoundly affected by grassland types and simulated climate changes (Table 1). The OTUs' percentage of fungal rare biosphere (FRB) and the relative abundance of BRB and FRB were affected neither by grassland types nor by experiment treatments of climate change (Table 1). The OTUs' percentage of BRB was higher in the AM ($95.1 \pm 0.1\%$) than in the AS ($94.7 \pm 0.1\%$) (Table 1, Fig. 2a). In terms of the relative abundance, the BRB accounted for approximately 40% of the bacterial community in the AM and the AS (Fig. 2c). The FRB accounted for 80–90% of the OTUs of the fungal community in the alpine grassland

Table 1

Effects of grassland types (GT) and experimental treatments (ET) on OTUs' percentage and relative abundance of bacterial rare biosphere (BRB) and fungal rare biosphere (FRB). *F* value is shown.

	Percentage of OTUs		Relative abundance	
	BRB	FRB ^a	BRB	FRB
GT	15.25***	0.55	1.70	3.42
ET	3.81*	2.75	2.52	2.78
GT*ET	1.41	0.50	0.49	0.23

* $p < 0.05$.

*** $p < 0.001$.

^a a sine transformation was conducted to satisfy the demand of homogeneity of variance.

soils (Fig. 2b). The relative abundance of the FRB was less than 10% of the fungal communities in the AM and the AS (Fig. 2d).

3.2. Specific taxa of soil microbes under climate changes

In the AM, the OTUs number of specific bacterial taxa (SBT) was the highest under the SW treatment, whereas their numbers under the VW and ER treatments were lower than under the CK treatment (Fig. 3a). In the AS, the OTUs number of SBT was the highest under the CK treatment. The SW, VW and ER treatments reduced the OTUs number of SBT (Fig. 3a).

In the AM, the OTUs' number of specific fungal taxa (SFT) was increased by the SW, VW and ER treatments. The highest OTUs' number of SFT was detected under the SW treatment (Fig. 3b). In the AS, the OTUs' number of SFT was reduced by the VW and ER treatments (Fig. 3b).

According to 0.1% threshold of relative abundance, all SBT belonged to the BRB (Table 2). Over 90% of SFT belonged to the FRB (Table 2). The relative abundance of both SBT and SFT were less than 1% in soils of the AM and AS (Fig. 2c, d).

3.3. Soil nutrients and plant biomass under climate changes

Soil TN, TP, available nitrogen ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) and AP varied significantly between the AM and the AS, while they were not significantly affected by simulated warming or enhanced rainfall (Table 3). The contents of soil TN and $\text{NO}_3\text{-N}$ were higher in the AM than those in the AS. The contents of soil TP and $\text{NH}_4\text{-N}$ were higher in the AS than those in the AM (Table 4).

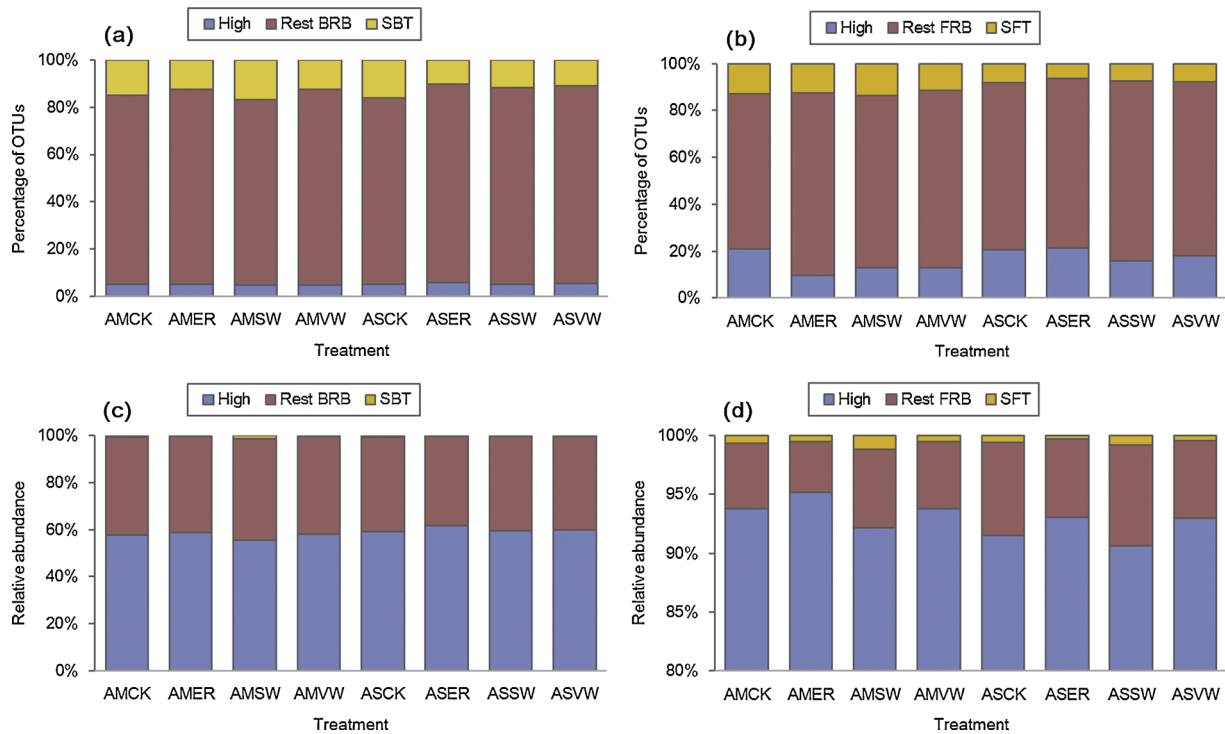


Fig. 2. The rare biosphere and specific taxa of soil microbes under the treatments of control (CK), enhanced rainfall (ER), stable warming (SW) and variable warming (VW) in an alpine meadow (AM) and alpine steppe (AS). (a) the percentages of OTUs of soil specific bacteria taxa (SBT), the rest bacteria rare biosphere (Rest BRB) and the high abundance bacteria taxa (H); (b) the percentages of OTUs of soil specific fungi taxa (SFT), the rest bacteria rare biosphere (Rest FRB) and other high abundance fungi taxa (H); (c) relative abundances of SBT, the Rest BRB and high abundance bacteria taxa; (d) relative abundances of SFT, the Rest FRB and other high abundance fungi taxa.

3.4. Effects of RB on soil nutrients and plant biomass

The χ^2 value of the RB's model and the abundant species' model were 22.31 and 12.05 ($p > 0.05$), respectively. In addition, for both of these two models, the comparative fit index (CFI) was 1.00 and the root mean square error of approximation (RMSEA) was 0.00. The abundance of SBT was positively related to the abundance of SFT (Fig. 4a). The rest BRB (i.e., the BRB that excluding SBT) and the SFT negatively affected the contents of soil $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in a direct way, respectively (Fig. 4a). The rest FRB, i.e., the FRB that excluding the SFT, positively affected the contents of soil $\text{NH}_4\text{-N}$ (Fig. 4a). Moreover, the rest FRB and SFT affected AGB by affecting the contents of soil $\text{NH}_4\text{-N}$ (Fig. 4a). Soil TP positively affected $\text{NH}_4\text{-N}$ and AP directly, affecting the AGB indirectly (Fig. 4a). Soil TN affected AGB in both direct and indirect ways. Directly, the soil TN negatively affected AGB strongly. Indirectly, the soil TN affected AGB by positively affecting the contents of soil $\text{NO}_3\text{-N}$ and AP, which promoted the AGB (Fig. 4a). Soil $\text{NO}_3\text{-N}$ and AP

Table 2

The threshold of “rare biosphere” and the percentage of specific OTUs belonging to “rare biosphere” in an alpine meadow (AM) and alpine steppe (AS) under the treatments of control (CK), enhanced raining (ER), stable warming (SW) and variable warming (VW) on the QTP. Mean $\pm$ S.E. value is shown.

Grassland Types	Treatments	0.1% threshold of abundance		Specific OTUs below threshold (%)	
		bacteria	fungi	bacteria	Fungi
AM	CK	63.9 $\pm$ 2.9	37.9 $\pm$ 1.9	100	94.3
	ER	62.9 $\pm$ 2.5	36.4 $\pm$ 1.4	100	97.0
	SW	58.3 $\pm$ 2.2	38.0 $\pm$ 1.4	100	94.8
	VW	67.5 $\pm$ 2.0	35.9 $\pm$ 1.1	100	96.7
AS	CK	67.8 $\pm$ 2.0	34.9 $\pm$ 2.0	100	99.2
	ER	62.0 $\pm$ 2.7	36.5 $\pm$ 1.7	100	95.4
	SW	61.5 $\pm$ 2.8	36.4 $\pm$ 1.4	100	89.7
	VW	66.2 $\pm$ 2.8	34.6 $\pm$ 1.11	100	97.1

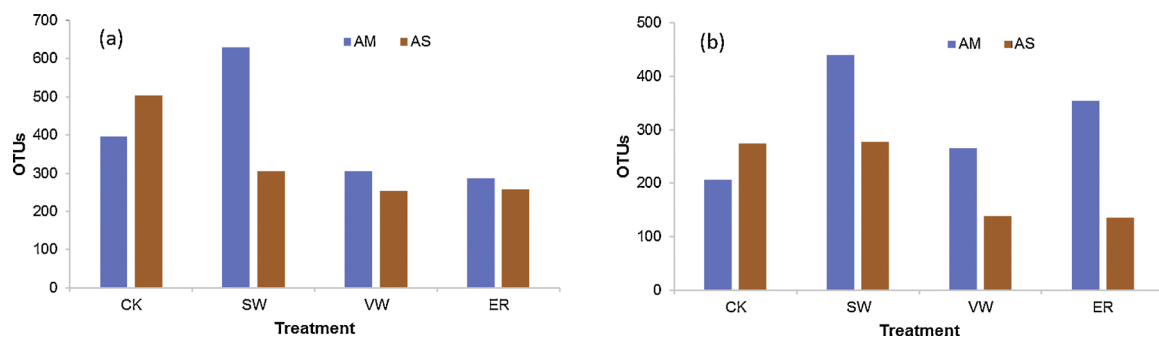


Fig. 3. OTUs' number of specific taxa of soil bacteria (a) and soil fungi (b) under control (CK), stable warming (SW), variable warming (VW) and enhanced rainfall (ER) in an alpine meadow (AM) and alpine steppe (AS). There were no S.E. for the number of OTUs, because the specific taxa were defined as the unique taxa that detected in some treatment. For each grassland type, the replicated samples of each treatment were analyzed together.

Table 3

Effects of grassland types (GT) and experimental treatments (ET) on soil total nitrogen (TN), total phosphorus (TP), available nitrogen ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) and available phosphorus (AP) and aboveground biomass (AGB) of plant communities. The F value and its significance are shown.

	TN (g/kg)	TP (g/kg)	$\text{NO}_3\text{-N}$ (mg/kg)	$\text{NH}_4\text{-N}$ (mg/kg)	AP (mg/kg)	AGB (g/m ²)
GT	13.47***	39.94***	10.67***	50.42***	2.84	3.76
ET	0.53	0.84	2.79	0.24	0.32	2.71
GT*ET	0.69	1.63	0.84	1.81	0.69	0.85

*** $p < 0.001$.

positively affected AGB of plant communities in direct ways, while soil $\text{NH}_4\text{-N}$ affected the AGB by affecting the content of soil $\text{NO}_3\text{-N}$ (Fig. 4a).

The summed effects and the synthesize of direct and indirect effects, of soil RB on soil TN, TP, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and AP and plant AGB were different. The FRB played a more important role than the BRB in regulating the contents of soil nutrients. And the SFT and the rest FRB played opposite roles in affecting the contents of soil nutrients (Table 5). In the model of RB, the variations of soil $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, AP, TN, TP and AGB were explained by 58%, 65%, 64%, 12%, 6% and 44%, respectively (Fig. 4a).

The sole significant path was detected between the abundance of abundant fungi and the content of soil total nitrogen in the model of abundant species (Fig. 4b). The effects of the abundance of abundant bacteria and abundant fungi on the contents of soil total nitrogen, total phosphorus, $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were opposite (Table 5). In the model of abundant species, the variations of soil $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, AP, TN, TP and AGB were explained by 48%, 49%, 61%, 22%, 12% and 42%, respectively (Fig. 4b). The variations of soil available nitrogen ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) explained by abundant species were much lower than by the RB (Fig. 4).

4. Discussion

The mineralization of nutrients in soils is an important segment of biogeochemical cycle on the Earth (Bardgett, 2005). It is suggested that climate changes, e.g. warming, would change the storage of soil nitrogen, which could regulate climate changes in return (Stocker et al., 2013; Bradford et al., 2016). However, we found in the present study that both warming (including SW and VW) and ER did not significantly affect soil total nitrogen, total phosphorus, available nitrogen and available phosphorus in alpine grassland ecosystems of the QTP. Our results were consistent with the results obtained from both short (2 years) and long-term (10 years) warming experiments on an alpine meadow of the QTP (Wang et al., 2014). This suggested that the total nutrients and available nutrients in alpine grassland soils of the QTP were stable in response to climate change. This might be attributed to two ecological processes. On the one hand, climate changes, e.g. warming, could enhance the degradation of nutrient materials in soils, which performed as the growing primary productivity of plant community and the increase of soil respiration (Piao et al., 2012; Gao et al., 2016). On the other hand, warming could significantly improve litter inputs and the degradation rate of litter mass, which brought lots of nitrogen into soils (Luo et al., 2010; Rui et al., 2011).

These two ecological processes might be largely controlled by soil

microbes, which occupy an important position in the tree of life and play crucial roles in element cycling (Hug et al., 2016) and in responses of ecosystems to climate changes (Singh et al., 2010). Most microbes are members of the RB, characterized as low abundance but high diversity, and are treated as the seed bank of microbes (Lynch and Neufeld, 2015). Although the RB has been widely detected in aquatic ecosystem (Sogin et al., 2006; Galand et al., 2009; Sauret et al., 2014), few literatures have documented RB in territorial ecosystems. In the present study, we found there was a substantial microbial RB in alpine grassland soils, i.e. the BRB contributed over 95% and the FRB contributed over 80% OTUs to microbial communities. We also found that neither warming nor enhanced rainfall significantly changed the relative abundance of BRB and FRB. This implied that the RB of soil microbes was robust under climate change conditions in alpine grassland ecosystems of the QTP. Therefore, we rejected the first hypothesis of this study, i.e. the abundance of bacterial and fungal RB would be changed by warming or enhanced rainfall.

The RB could greatly affect the functions (such as nutrient cycling) of an ecosystem directly (Lynch and Neufeld, 2015). In the present study, we found that the RB of soil microbes, i.e., RBR and FRB, explained many more variations in the contents of soil available nitrogen ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) than the high abundance taxa of soil microbes under climate change conditions on the QTP. The content of soil $\text{NO}_3\text{-N}$ was mainly affected by bacterial RB, while the content of soil $\text{NH}_4\text{-N}$ was significantly affected by fungal RB. Furthermore, we found that the specific fungi taxa and the remaining fungi RB (i.e. the fungi RB excluding specific fungi taxa) played opposite roles in regulating soil $\text{NH}_4\text{-N}$. These may suggest that the underground microbial RB plays an important role in regulating soil available nitrogen of alpine grassland ecosystems under climate changes.

For phosphorus, it suggested that soil fungi community contained many species that helped promote the availability of soil phosphorus in farmland soils (Li et al., 2016). However, we did not detect any significant paths between the abundance of soil fungi taxa (both the rare and the abundant) and the contents of soil phosphorus (both the total and the available) in the present study. This inconformity might be caused by: 1) the differences of fungi communities between farmland ecosystems and alpine grassland ecosystems; 2) the abundance of these phosphorous mineralization related fungi taxa is low in natural ecosystems so that their effects could not be detected by statistical analyses (such as the path analysis) based on field surveys; and 3) the simulated climate changes eliminated these taxa from fungal communities, which should be considered in future studies.

In addition, it is reported that environment-specific microbial species play critical roles in mitigating the negative impacts of environmental changes (Spiro and Guest, 1987; Merrick and Edwards, 1995; Yang et al., 2013; Hua et al., 2014). In this study, we identified specific taxa that can only be detected under some treatments, i.e. the SBT and SFT. We found that nearly all of them were member of the RB. Our findings provided new evidences from alpine ecosystems for the fact that RB contributes to discriminating environmental samples, due to the existence of specific taxa (Youssef et al., 2009; Gobet et al., 2012; Lynch and Neufeld, 2015). Moreover, the path analysis in the present study revealed that the content of soil $\text{NH}_4\text{-N}$ was strongly affected by the SFT, which means the specific taxa could play roles in affecting soil available nitrogen in alpine grassland ecosystems under climate

Table 4

Soil nutrients (0–15 cm) and plant aboveground biomass (AGB) in an alpine meadow (AM) and alpine steppe (AS) of the QTP. Mean $\pm$ S.E. is shown.

	TN (g/kg)	TP (g/kg)	$\text{NO}_3\text{-N}$ (mg/kg)	$\text{NH}_4\text{-N}$ (mg/kg)	AP (mg/kg)	AGB (g/m ²)
AM	4.17 $\pm$ 0.21a	0.52 $\pm$ 0.01b	24.93 $\pm$ 1.77a	2.60 $\pm$ 0.39b	10.07 $\pm$ 0.53a	83.75 $\pm$ 7.71a
AS	3.20 $\pm$ 0.14b	0.65 $\pm$ 0.02a	15.03 $\pm$ 2.85b	8.02 $\pm$ 0.66a	11.61 $\pm$ 0.66a	104.45 $\pm$ 8.87a

Different letters mean significant differences at the level of $\alpha = 0.05$.

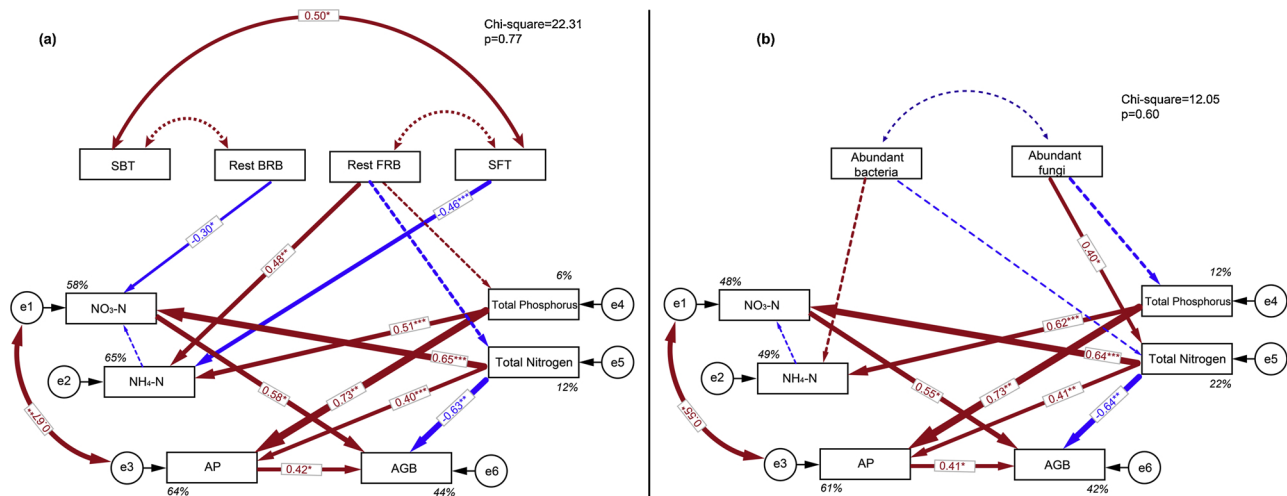


Fig. 4. Effects of “rare biosphere” (a) and abundant microbial taxa (b) on soil available nutrients ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and available phosphorus, i.e. AP), total nitrogen, total phosphorus and aboveground biomass (AGB). The rare biosphere of soil bacteria contained all the specific bacteria taxa (SBT) and the rest rare bacteria (Rest BRB). Fungal rare biosphere (FRB) contained some specific fungi taxa (SFT) and the rest part of the FRB (Rest FRB). The red line represents positive effects and the blue line means negative effects. The dashed line represents non-significant effects, but they are helpful to improve model quality. The number on the one-way arrow is the effect coefficient, and on the two-way arrow is the correlation coefficient. The italic percentage near the corner of each endogenous variable is the explained variations. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 5

Standardized total effects of rare biosphere and abundant taxa on soil nutrients and plant aboveground biomass. R-BRB: the rest bacterial rare biosphere, i.e., the rare biosphere of bacteria excluding specific bacteria taxa; SFT: specific fungi taxa; R-FRB: the rest fungal rare biosphere, i.e., the rare biosphere of fungi excluding the SFT; AP: available phosphorus; TN: total nitrogen; TP: total phosphorus; AGB: aboveground biomass.

	Rare biosphere			Abundant taxa	
	R-BRB ^a	R-FRB ^b	SFT	Abundant bacteria	Abundant fungi
TN	0.00	−0.35	0.00	−0.20	0.40
TP	0.00	0.25	0.00	0.00	−0.34
$\text{NO}_3\text{-N}$	−0.31	−0.34	0.08	−0.18	0.29
$\text{NH}_4\text{-N}$	0.00	0.61	−0.46	0.28	−0.21
AP	0.00	0.05	0.00	−0.08	−0.09
AGB	−0.18	0.04	0.05	−0.01	−0.13

changes. The regulating processes of the RB on soil available nitrogen may help to stabilize the aboveground biomass of plant communities under the stress of climate change in the QTP, which is characterized by a shortage of available nitrogen. These evidences can partly support the second hypothesis of this study, i.e., the soil microbial RB could help to stabilize the aboveground biomass of alpine grasslands by regulating soil available nitrogen.

5. Conclusions

In conclusion, the relative abundance of the RB of soil bacteria and fungi did not change after 3-year simulated warming and enhanced rainfall. The robust soil microbial RB played important roles in regulating the content of soil available nitrogen in alpine grassland ecosystems and thus helped the ecosystems to cope with climate changes on the QTP.

Conflict of interest

The authors declare no conflict of interest in relation to the submitted work.

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References

- Bardgett, R.D., 2005. *The Biology of Soil: A Community and Ecosystem Approach*. Oxford University Press, New York, pp. 57–118.
- Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and below-ground food webs: how plant responses to foliar herbivory influence soil organism. *Soil Biol. Biochem.* 30, 1867–1878.
- Bews, J.W., 1930. The world's grasses: their differentiation, distribution, economics and ecology. *Nature* 125, 119–121.
- Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A., Crowther, T.W., 2016. Managing uncertainty in soil carbon feedbacks to climate change. *Nat. Clim. Change* 6, 751–758.
- Chapin III, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., et al., 2000. Consequences of changing biodiversity. *Nature* 405, 234–242.
- Chen, H., Zhu, Q., Peng, C., Wu, N., Wang, Y., Fang, X., et al., 2013. The impacts of climate change and human activities on biogeochemical cycles on the Qinghai-Tibetan Plateau. *Glob. Change Biol. Bioenergy* 19, 2940–2955.
- Decaens, T., 2010. Macroecological patterns in soil communities. *Glob. Ecol. Biogeogr.* 19, 287–302.
- Ding, M.J., Zhang, Y.L., Sun, X.M., Liu, L.S., Wang, Z.F., Bai, W.Q., 2013. Spatio-temporal variation in alpine grassland phenology in the Qinghai-Tibetan Plateau from 1999 to 2009. *Chin. Sci. Bull.* 58, 396–405.
- Dong, S.K., Sherman, R., 2015. Enhancing the resilience of coupled human and natural systems of alpine rangelands on the Qinghai-Tibetan Plateau. *Rangeland J.* 37, i–iii.
- Dong, S.K., Wen, L., Li, Y.Y., Wang, X.X., 2015. Plant-Soil Interface Progress in Ecological Restoration of Degraded Alpine Grasslands on Qinghai-Tibetan Plateau. Science Press, Beijing, pp. 10–13.
- Galand, P.E., Casamayor, E.O., Kirchman, D.L., Lovejoy, C., Karl, D.M., 2009. Ecology of the rare microbial biosphere of the Arctic Ocean. *Proc. Natl. Acad. Sci. U. S. A.* 52, 22427–22432.
- Ganjurjav, H., Gao, Q.Z., Gornish, E.S., Schwartz, M.W., Liang, Y., Cao, X.J., et al., 2016. Differential response of alpine steppe and alpine meadow to climate warming in the central Qinghai-Tibetan Plateau. *Agr. Forest Meteorol.* 223, 233–240.
- Gao, Q.Z., Zhu, W., Schwartz, M.W., Ganjurjav, H., Wan, Y., Qin, X., et al., 2016. Climate change controls productivity variation in global grasslands. *Sci. Rep.* 6, 26958.
- Gaston, K.J., 2000. Global patterns in biodiversity. *Nature* 405, 220–227.
- Giovannoni, S.J., Stingl, U., 2005. Molecular diversity and ecology of microbial plankton. *Nature* 437, 343–348.

- Gobet, A., Boer, S.I., Huse, S.M., van Beusekom, J.E.E., Quince, C., Sogin, M.L., et al., 2012. Diversity and dynamics of rare and of resident bacterial populations in coastal sands. *ISME J.* 6, 542–553.
- Harris, R.B., 2010. Rangeland degradation on the Qinghai–tibetan plateau: a review of the evidence of its magnitude and causes. *J. Arid Environ.* 74, 1–12.
- Horner-Devine, M.C., Carney, K.M., Bohannan, B.J.M., 2004. An ecological perspective on bacterial biodiversity. *Proc. R Soc. Lond. B* 271, 113–122.
- Hua, Z.S., Han, Y.J., Chen, L.X., Liu, J., Hu, M., Li, S.J., et al., 2014. Ecological roles of dominant and rare prokaryotes in acid mine drainage revealed by metagenomics and metatranscriptomics. *ISME J.* 7, 1–15.
- Hug, L.A., Baker, B.J., Anantharaman, K., Brown, C.T., Probst, A.J., Castelle, C.J., et al., 2016. A new view of the tree of life. *Nat. Rev. Microbiol.* 1, 16048.
- IPCC, 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.
- Jonsson, M., Wardle, D.A., 2010. Structural equation modeling reveals plant–community drivers of carbon storage in boreal forest ecosystems. *Biol. Lett.* 6, 116–119.
- Klein, J.A., Harte, J., Zhao, X.Q., 2007. Experimental warming, not grazing, decreases rangeland quality on the Tibetan Plateau. *Ecol. Appl.* 17, 541–557.
- Li, Z., Bai, T., Dai, L., Wang, F., Tao, J., Meng, S., et al., 2016. A study of organic acid production in contrasts between two phosphate solubilizing fungi: *penicillium oxalicum* and *Aspergillus niger*. *Sci. Rep.* 6, 25313.
- Llorens-Marès, T., Yooshep, S., Goll, J., Hoffman, J., Vila-Costa, M., Borrego, C.M., et al., 2015. Connecting biodiversity and potential functional role in modern euxinic environments by microbial metagenomics. *ISME J.* 9, 1648–1661.
- Luo, C.Y., Xu, G., Chao, Z., Wang, S., Lin, X., Hu, Y., et al., 2010. Effect of warming and grazing on litter mass loss and temperature sensitivity of litter and dung mass on the Tibetan plateau. *Glob. Change Biol. Bioenergy* 16, 1606–1617.
- Lynch, M.D.J., Bartram, A.K., Neufeld, J.D., 2012. Targeted recovery of novel phylogenetic diversity from next-generation sequence data. *ISME J.* 6, 2067–2077.
- Lynch, M.D.J., Neufeld, J.D., 2015. Ecology and exploration of the rare biosphere. *Nat. Rev. Microbiol.* 13, 217–229.
- McGrady-Steed, J., Harris, P.M., Morin, P.J., 1997. Biodiversity regulates ecosystem predictability. *Nature* 390, 162–165.
- Merrick, M., Edwards, R., 1995. Nitrogen control in bacteria. *Microbiol. Rev.* 59, 604–622.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, C.A., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.
- Pester, M., Bittner, N., Deevong, P., Wagner, M., Loy, A., 2010. A ‘rare biosphere’ microorganism contributes to sulfate reduction in a peatland. *ISME J.* 4, 1591–1602.
- Piao, S.L., Tan, K., Nan, H., Ciais, P., Fang, J., Wang, T., et al., 2012. Impacts of climate and CO₂ changes on the vegetation growth and carbon balance of Qinghai–Tibetan Plateau grasslands over the past five decades. *Global Planet. Change* 98, 73–80.
- Rosling, A., Cox, F., Martinez, C., Ihrmark, K., Grelet, G.A., Lindahl, B.D., et al., 2011. Archaeorhizomycetes: unearthing an ancient class of ubiquitous soil fungi. *Science* 333, 876–879.
- Rui, Y.C., Wang, S., Xu, Z., Wang, Y., Chen, C., Zhou, X., et al., 2011. Warming and grazing affect soil labile carbon and nitrogen pools differently in an alpine meadow of the Qinghai–Tibet Plateau in China. *Int. J. Soil Sediment Water* 11, 903–914.
- Sauret, C., Séverina, T., Vétion, G., Guigue, C., Goutx, M., Pujo-Pay, M., et al., 2014. Rare biosphere” bacteria as key phenanthrene degraders in coastal seawaters. *Environ. Pollut.* 194, 246–253.
- Shade, A., Jones, S.E., Caporaso, J.G., Handelsman, J., Knight, R., Fierer, N., et al., 2014. Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. *MBio* 5 e01371–14.
- Singh, B.K., Bardgett, R.D., Smith, P., Reay, D.S., 2010. Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nat. Rev. Microbiol.* 8, 779–790.
- Sogin, M.L., Morrison, H.G., Huber, J.A., Welch, D.M., Huse, S.M., Neal, P.B., et al., 2006. Microbial diversity in the deep sea and the underexplored “rare biosphere”. *Proc. Natl. Acad. Sci. U. S. A.* 103, 12115–12120.
- Spiro, S., Guest, J.R., 1987. Regulation and over-expression of the *fmr* gene of *Escherichia coli*. *J. Gen. Microbiol.* 133, 3279–3288.
- Stocker, B.D., Roth, R., Joos, F., Spahni, R., Steinacher, M., Zaehle, S., et al., 2013. Multiple greenhouse-gas feedbacks from the land biosphere under future climate change scenarios. *Nat. Clim. Change* 3, 666–672.
- Tilman, D., Reich, P.B., Knops, J.M., 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* 441, 629–632.
- Wang, X.X., Dong, S.K., Gao, Q.Z., Zhou, H.K., Liu, S.L., Su, X.K., et al., 2014. Effects of short-term and long-term warming on soil nutrients, microbial biomass and enzyme activities in an alpine meadow on the Qinghai–Tibet Plateau of China. *Soil Biol. Biochem.* 76, 140–142.
- Wei, C., Yu, Q., Bai, E., Lv, X., Li, Q., Xia, J., et al., 2013. Nitrogen deposition weakens plant–microbe interactions in grassland ecosystems. *Glob. Change Biol. Bioenergy* 19, 3688–3697.
- Xu, Z.X., Gong, T.L., Li, J.Y., 2008. Decadal trend of climate in the Tibetan Plateau—regional temperature and precipitation. *Hydrol. Process.* 22, 3056–3065.
- Yang, Y.F., Gao, Y., Wang, S.P., Xu, D.P., Yu, H., Wu, L.W., et al., 2013. The microbial gene diversity along an elevation gradient of the Tibetan grassland. *ISME J.* 8, 430–440.
- Youssef, N., Sheik, C.S., Krumholz, L.R., Najjar, F.Z., Roe, B.A., Elshahed, M.S., 2009. Comparison of species richness estimates obtained using nearly complete fragments and simulated pyrosequencing-generated fragments in 16S rRNA gene-based environmental surveys. *Appl. Environ. Microb.* 75, 5227–5236.
- Zhang, Y., Dong, S.K., Gao, Q.Z., Liu, S.L., Zhou, H.K., Ganjurjav, H., et al., 2016. Climate change and human activities altered the diversity and composition of soil microbial community in alpine grasslands of the Qinghai–Tibetan Plateau. *Sci. Total Environ.* 562, 353–363.